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The HIV envelope protein, gp120 binds to the CD4 cell surface protein and thereby the virus enters T4 helper lymphocytes. This process of virus entry might be associated with the presentation of unique epitopes of the gp120 that become revealed only as a result of virus binding to its target cell. The purpose of this research has been to identify cryptic epitopes that become accessible due to induced conformational changes that result from that complex formation of gp120 with CD4. Thus CD4/gp120 complexes have been used to immunize mice and monoclonal antibodies have been generated. At least 7 antibodies have been identified as having preferred affinity for the above complex. These antibodies are now being characterized. They represent 3 distinct epitopes as has been determined by competitive ELISA assays. Moreover, using a syncytium assay they have been evaluated for their neutralizing activity.

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1 June 94
Date

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INTRODUCTION:

The development of an effective vaccine for the prevention of AIDS is a major goal that has been the focus of intensive research for the past decade. Efforts have focused around the viral envelope glycoprotein gp120 which is noncovalently attached to the particle via the membrane-associated gp41.

The structure of gp120 has been extensively analyzed and the glycomoieties, the disulfide arrangements [1] and variable *versus* conserved domains have been defined [2]. The most prominent region of this protein is the Primary Neutralizing Domain (PND) that consists of the V3 loop (residues 266-301, [3]). Antibodies to this epitope have been found to be neutralizing in a highly strain-selective manner [4]. Nonetheless, there are regions within this loop that can elicit broad spectrum neutralization [5, 6]. A second target within the gp120 is the area involved in CD4 recognition [7]. Obviously, antibodies that bind this region are anticipated to interfere with the virus association to its target cells and thus prevent infection. This epitope is believed to be complex being constructed from discontinuous segments of the gp120. A desired objective would be to produce a subunit vaccine that would provide broad spectrum protection against HIV infection. Unfortunately, neither the V3 loop nor the CD4 binding domains of gp120 appear to satisfy this goal.

Therefore, the search for novel more effective epitopes for the production of subunit vaccines is still extremely important and relevant. The strategy proposed in this study has been to identify new aspects of the gp120 molecule that could serve this purpose. The approach adopted here is based on the assumption that gp120 can assume a variety of *conformations*. In doing so gp120 would thus present different aspects of itself during various phases of the disease. For example, gp120 when associated with gp41 may be quite distinct from soluble gp120 or the CD4 bound protein. The notion that the envelope undergoes conformational rearrangements is supported by the fact that the N terminal domain of gp41 becomes available for membrane fusion only after the gp120 attaches to CD4 [8].

Recently, this line of thought has been further elaborated upon in studies that focus on the idea that after the binding of gp120 to CD4 there must exist a series of events that are related to viral *entry* rather than binding *per se*. Celada et al [9] and Healey et al [10] both focus on this point and develop mAbs against CD4 that have neutralizing activity yet are by definition not of the classical type of Leu3A or OKT4A namely, the new antibodies do not interfere with the complex formation.

During the course of this research program we have performed a series of experiments in which we have immunized mice with recombinant gp120 in presentations not commonly used. Thus for example we have immunized mice with the CD4/gp120 complex and isolated monoclonal antibodies that show a distinct preference for the complexed configuration of its components.

BODY:

Materials:

Recombinant gp120 produced in a baculovirus expression system was purchased from American Bio-Technologies. Recombinant soluble CD4 (produced in CHO cells) as well as a anti-V3 loop mAbs (NEA-9305) were obtained from DuPont. The rest of the reagents used were standard and of analytical grade.

Methods:

V8 protease digests: Recombinant gp120 (10µg/ml) was incubated 24-48 hours in 50mM NH_4HCO_3 pH7.8/0.05% SDS/1µg *Staphylococcus aureus* V8 protease at 37°C. Addition of ß-mercaptoethanol (5%) to the reaction generated a unique set of fragments.

Protein blot analyses: Intact or proteolysed protein fragments were separated on SDS-polyacrylamide gels as previously described and transferred electrophoretically in an apparatus that generates a gradient electric field to compensate for differences in molecular mass of the peptides being blotted [11]. Either nitrocellulose or charged modified nylon membranes were used as the blotting matrix. Blots were quenched either with 1% milk solutions in 50mM Tris buffered saline pH7.5 (TBS) or 5% hemoglobin solutions in the same. Probes such as biotinylated lectins or mAbs were routinely diluted in a quenching solution and their presence revealed by using enzyme conjugated second reagents and standard detection reactions (for a general review on the above blotting procedures see [12]).

ELISA assays: Costar EIA/RIA 96 well plates (N3590) were coated with 50µl of gp120/CD4 complex (5µg gp120:2.5µg CD4/ml) or gp120 (5µg/ml) or CD4 (5µg/ml) in TBS overnight at 4°C. The plates were then washed in TBS and blocked with 3% BSA in TBS for 1 hour at room temperature (RT). The wells were rinsed and the mAbs at various dilutions in 0.3%BSA/TBS were added to them and incubated at RT for 2-3 hours. The wells were then washed with TBS and the second antibody (alkaline phosphatase conjugated goat anti-mouse antibody [Sigma, A-0162]) was added (1:1000 in 0.3%BSA/TBS) and incubated for 1 hour at RT. After washing the wells they were reacted with p-nitrophenyl phosphate (1mg/ml in 1M diethanolamine buffer pH9.8 / 0.5mM $MgCl_2$) and read at 405nm.

Production of monoclonal antibodies: The production of mAbs was performed using standard procedures immunizing the mice (Balb/C) first in complete Freund's adjuvant and boosted with in-complete adjuvant. The mice were allowed to rest 3 weeks before their i.v. boost and splenectomy followed three days later. NS-1 myeloma cells were used for PEG induced fusion and HAT medium for selection. Clones were obtained by limited dilutions [13].

mAb purification: mAbs were isolated from ascites fluids taken from pristane primed mice. The standard caprylic acid followed by ammonium sulfate precipitation procedure was used [13].

Biotinylation of mAbs: mAbs (~1mg/ml) were dialysed overnight against 0.1M $NaHCO_3$ (pH was not adjusted). Biotinamidocaproate N-hydroxysuccinimide ester (BNS, [Sigma B-2643]) was added (3µg/100µg protein) and incubated for 4 hours at RT, followed by overnight dialysis against TBS.

Radioiodination of mAbs: 100µg (100µl of PBS) of a specific mAb was incubated with 10µl chloramine-T (2.5 mg/ml) to which 0.5mCi ^{125}I (10µl) were added. The mixture was incubated on ice for 5 min. and the reaction stopped with sodium metabisulfite. Then the iodine was separated from the labeled protein by G-25 sephadex chromatography.

Binding of mAbs to cell surface CD4: 10^6 CEM cells were incubated in BSA-prequenched wells of a 96-well microtiter plate (Costar). The cells were incubated with gp120 (10µg/ml) for 2hr at RT as indicated. Various concentrations of radioiodinated mAbs were added to the cells and incubated overnight at 4°C. The following morning the cells washed in PBS and the amount of bound mAb was quantified using a Pakard Cobra gamma counter.

Syncytium assay: The formation of syncytia between vaccinia infected BSC1 cells and CEM cells was performed essentially as was described by Ashorn et al. [14] In principle, BSC1 cultures were infected with recombinant vaccinia (5pfu/ml) expressing cell surface gp120 (VPE16 provided by Dr. Bernie Moss, NIH). These were then mixed with CEM cells in the presence of variable amounts of mAbs and incubated for different times. The degree of syncytia formation was monitored and thus the extent of neutralization potential for the various mAbs was estimated.

Results:

Characterization of V8 proteolyzed gp120: Digestion of gp120 with *S. aureus* V8 protease produces 5 major fragments detectable on western blots probed with ConA (see schematic summary in Figure 1).

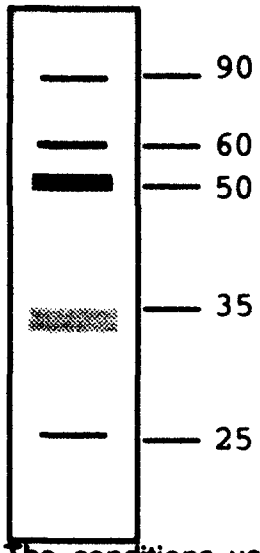


Figure 1: Schematic Diagram of V8 Proteolyzed gp120.

Western blots of V8 proteolyzed gp120 were probed with anti V3 mAbs, ConA or sCD4. To the left is a summary of the bands which can be detected. Depending on the degree of digestion and the probed used the presence and the intensity of each band can vary.

The conditions used (see Methods) were found to generate reasonably reproducible patterns although in view of the fact that the simple sum of the molecular weights of the fragments yields a total mass of over 250kDa the pattern received can only be a set of overlapping fragments rather than a true terminal digest. The reasons for this are unclear. More aggressive denaturation might however, lead to over digestion and the generation of numerous unresolved small fragments. Western blots of transferred V8 digested gp120 were analyzed with: ConA, commercial monoclonal anti gp120 antibodies, and with sCD4 itself. The signal for sCD4 overlay of proteolyzed gp120 is weak but reproducible. In view of the fact that it is believed that the critical CD4 binding domain is in the COOH aspect of gp120, combined with the response to the V3 loop antibody, we can postulate a gross map of the gp120 with respect to some of the fragments we resolve (Figure 2).

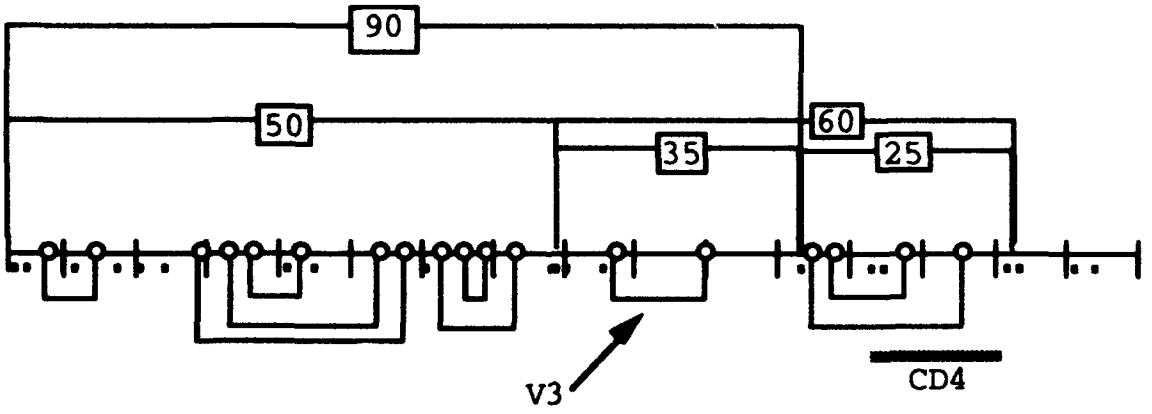


Figure 2: Map of gp120 V8 Fragments.

In the above diagram the circles indicate cys residues and the disulfide arrangements are given. The black dots indicate the approximate positions of the glutamic acid residues (V8 cleavage sites).

In figures 1 and 2 the 90kDa fragment contains the V3 loop but does not bind CD4. This band is lost after reduction with ß-mecaptoethanol and thus is probably the result of an early cleavage. The 60 kDa reacts with both anti V3 loop mAb and sCD4. The 50kDa does not bind CD4 nor anti V3 loop mAbs and is lost after reduction, indicating that it might be a series of small fragments held together via disulfide bridges. The 35 kDa fragment is the smallest containing the V3 loop and the reaction with anti V3 loop mAbs seems to intensify after reduction. The position of the 25kDa fragment can only be speculated.

Production of mAbs against V8 fragments of gp120: Three mice were injected with V8 cut gp120 and given a series of boosts. Initially, it appeared that only a weak response developed and thus additional boosts were given. As can be seen in Table 1 this increased the response of the mice against gp120.

TABLE 1: Analysis of Anti V8/gp120 Sera.

dilution of sera	I		II	
	gp120	V8/gp120	gp120	V8/gp120
1/50	674	-	1270	817
1/100	598	-	1220	624
1/200	310	-	1000	471
1/400	182	-	695	319
1/800	139	-	391	168
1/1600	-	-	229	93
1/3200	-	-	116	41
1/6400	-	-	77	31

The above table describes the response of one mouse that was immunized with gp120 V8 fragments. As can be seen, the titer is much improved in the second bleed.

The above mouse was selected and sacrificed on June 11, 1992. Its spleen contained $3x10^{-8}$ cells and these were fused in three aliquots with NS-1 myeloma cells and 2/3 of the cells were plated and processed as two separate fusions into a total of 13 96-well plates (the third aliquot was frozen down for future studies).

The hybridoma cultures were screened against gp120 and proteolysed gp120. Only five clones were found to be positive and one of these was lost in the process of cloning. The remaining four were found to be IgM of very low affinity and low titer in the ascites fluids obtained. It appears that the digested gp120 is a poor antigen and mice should be more aggressively immunized before new fusions are attempted. Unfortunately, in the mean time the remaining two mice that were immunized with the proteolyzed gp120 have died.

Production of CD4/gp120 complexes: CD4 and gp120 were used to generate their corresponding complex in a number of procedures. The sCD4/gp120 complex could be demonstrated via ELISA assays. In

principle, either gp120 or CD4 were plated and then the corresponding counter part was used to probe the plate. Subsequently, the plates were probed with either anti-gp120 or OKT4 followed by an alkaline phosphatase conjugated anti-mouse. Moreover, OKT4A was used to ascertain that the complex that was formed was, as expected, unable to bind this monoclonal (see Table 2). Finally, once conditions were established preparations of complex were made for immunizations.

TABLE 2: Analyses of gp120/CD4 Complexes.

gp120 µg/ml	OKT4A	OKT4	CD4 µg/ml	OKT4A	OKT4
10.0	27	640	5.0	206	729
5.0	23	773	2.5	135	492
2.5	27	313	1.25	42	291
1.25	29	116	0.60	22	138

The above table is an example of the analysis of the formation of gp120/CD4 complex on the ELISA plate used in this study. gp120 was applied to the wells using concentrations as indicated. Then CD4 (5µg/ml) was incubated with the gp120 and the wells were subsequently probed with the antibodies as indicated (numbers represent ODx10^{-3}). The right half of the table is a control in which CD4 was plated and the same antibodies were tested.

Production of anti-complex mAbs: Three mice were originally immunized against the complexed CD4/gp120. After the initial immunization two of the mice died and it was concluded that the MES (N-methylmorphiline) which is used as a preservative in the sCD4 might be toxic for the mice. A second set of three mice were then immunized with complex that had been extensively dialyzed. These mice and a remaining original mouse developed good responses against both CD4 and gp120 (see Table 3).

TABLE 3: Analysis of Anti Serum to CD4/gp120 Complex.

dilution of sera	gp120 5mg/ml	CD4 5mg/ml	CD4/gp120 5mg/ml
1/50	964	803	973
1/100	941	746	942
1/200	861	696	859
1/400	690	629	755
1/800	571	516	610
1/1600	462	463	502
1/3200	290	336	284
1/6400	299	297	250

The above depicts the response of a mouse that was immunized with the CD4/gp120 complex.

The mouse described in Table 3 was that with the highest titer and therefore was given an i.v. boost and was sacrificed for splenectomy and the fusion for hybridoma production. The spleen was found to extremely large (>4 x 10^8 cells were obtained). Therefore, 4 aliquots of ~10^8 cells were taken separately. Each was fused with NS-1 cells. Two fusions were processed in parallel (the remaining cells were frozen down for future studies). A total of 1170 clones were obtained and after 10 days of culture, the media were screened against CD4/gp120 complex in an ELISA assay. 147 clones were found positive and these were then re-screened against CD4/gp120

complex as well as CD4 and gp120 separately. Of the original clones only 81 continued to secrete antibodies and of these 15 were selected for future characterization. Of these 13 have proven to be stable and have been injected into mice for the successful production of ascites fluids.

Ascites fluids of all 13 mAbs described were produced and lyophylized and sent to Captain Tom VanCott. These were received and analyzed for their ability to bind CD4, gp120 and their complex. The mAbs were tested in ELISA assays, FACS analyses and BIAcore measurements. A collection of ten mAbs were identified as being interesting for further analyses (see Table 4).

Table 4:mAbs Produced Against gp120/CD4 Complex.

	mAb	subclass	gp120/CD4	gp120	CD4
1	CG-1	IgG1	+++	-	+/-
2	CG-4	IgG1	+++	+++	-
3	CG-7	IgG1	+++	-	+/-
4	CG-8	IgG1	+++	-	+/-
5	CG-9	IgG1	+++	-	++
6	CG-10	IgG1	+++	-	-
7	CG-25	IgG1	+++	-	++
8	CG-30	IgG1	+++	-	++
9	CG-40	IgG1	+++	+++	-
10	CG-76	IgG1	+++	-	+++

Results obtained in Tel Aviv corresponded well with those at Walter Reed. The antibodies were biotinylated and competitive ELISA assays were performed to determine epitope overlaps. In this manner six epitopes were defined (see Table 5).

Table 5: Epitope Classification by Competitive ELISA.

	1	4	7	8	9	10	25	30	40	76
1	+	-	+	+	-	-	-	-	-	-
4	-	+	-	-	-	-	-	-	-	-
7	+	-	+	+	-	-	-	-	-	-
8	+	-	+	+	-	-	-	-	-	-
9	-	-	-	-	+	-	+	+	-	-
10	-	-	-	-	-	+	-	-	-	-
25	-	-	-	-	+	-	+	+	-	-
30	-	-	-	-	+	-	+	+	-	-
40	-	-	-	-	-	-	-	-	+	-
76	-	-	-	-	-	-	-	-	-	+

Neutralization potential of the mAbs: The various mAbs were tested for their neutralizing activity using the syncytium assay described in the Methods. In this procedure BSC1 cells are infected with VPE16

recombinant vaccinia virus so that they express cell surface gp120. These cells are mixed with CEM cells and can therefore form syncytia. Syncytia formation is inhibited by the neutralizing antibodies. At least seven antibodies were found to be neutralizing to various degrees. Of particular interest was the fact that the mAb CG-10 which is complex specific by all the assays performed thus far in both labs, at Tel Aviv and at Walter Reed, was neutralizing, albeit with modest potency only. Of the mAbs described above five were extensively studied. These were: CG-4, CG-9, CG-10, CG-25 and CG-76.

These five mAbs represent four distinct epitopes only one of which is on gp120 and is not neutralizing. The other three are neutralizing to various degrees. As is demonstrated in Figure 3, when BSC1 cells infected with the recombinant vaccinia clone VEP16 are mixed with CEM cells, syncytia are readily formed with in a few hours (panel A) The CEM cells were either pre-incubated (5-12h) with mAbs (panels C, G, H, I) before infected BSC1 cells were added, or the mAbs were added simultaneously with the BSC1 cells (panels B, D, E, F). B: 10μg CG-25; C: 1μg CG-25; D & G: 1μg CG-9; E: 10μg CG-10; F: 1μg CG-76; H: 1μg CG-10; I: 1μg CG-76. As is clear from these experiments pre-incubation with the mAbs improves the neutralizing activity. Furthermore, CG-10 appears to be the least able to prevent syncytium formation however there is a marked difference between panels H and A, these results may also indicate that the epitope of CG-10 resides at least in part with in CD4..

Determination of affinities of mAbs for their antigens: In order to determine the extent of preference of the mAbs for complex over isolated CD4 or gp120 a series of Scatchard analyses were done. For this each mAb was first iodinated with ^{125}I and the Scatchards were performed using ELISA-plate immobilized antigens. As is demonstrated in Figure 4 and 5, mAbs CG-4 and CG-76 have no dramatic preference for the complex; mAb CG-10 in this assay has no affinity for the isolated antigens and binds to complex exclusively (Figure 6). Both mAbs CG-9 and CG-25 have 10 and 100 fold preference respectively for the complex over the isolated CD4 (Figure 7 and 8). Whereas these results had been repeated numerous times we did see variations in the extent of enhancement for these latter two clones which appeared to be dependent on the conditions of the assay used.

Therefore a series of experiments were conducted in which the assays used were of different plans as described here with:

1. Solid phase, application of the pre-prepared complex. In this ELISA assay the CD4 is complexed or not with gp120 in solution and then applied to the plate.

2. Solid phase, formation of the complex on the plate. In these experiments the CD4 is first applied to the plate and then gp120 is added at saturating concentrations thus forming the complex in the wells.

3. Solution phase. Here complex is made in solution and mAbs are incubated with the the complex in solution. Subsequently, the unbound mAb is quantified by standard ELISA against bound complex.

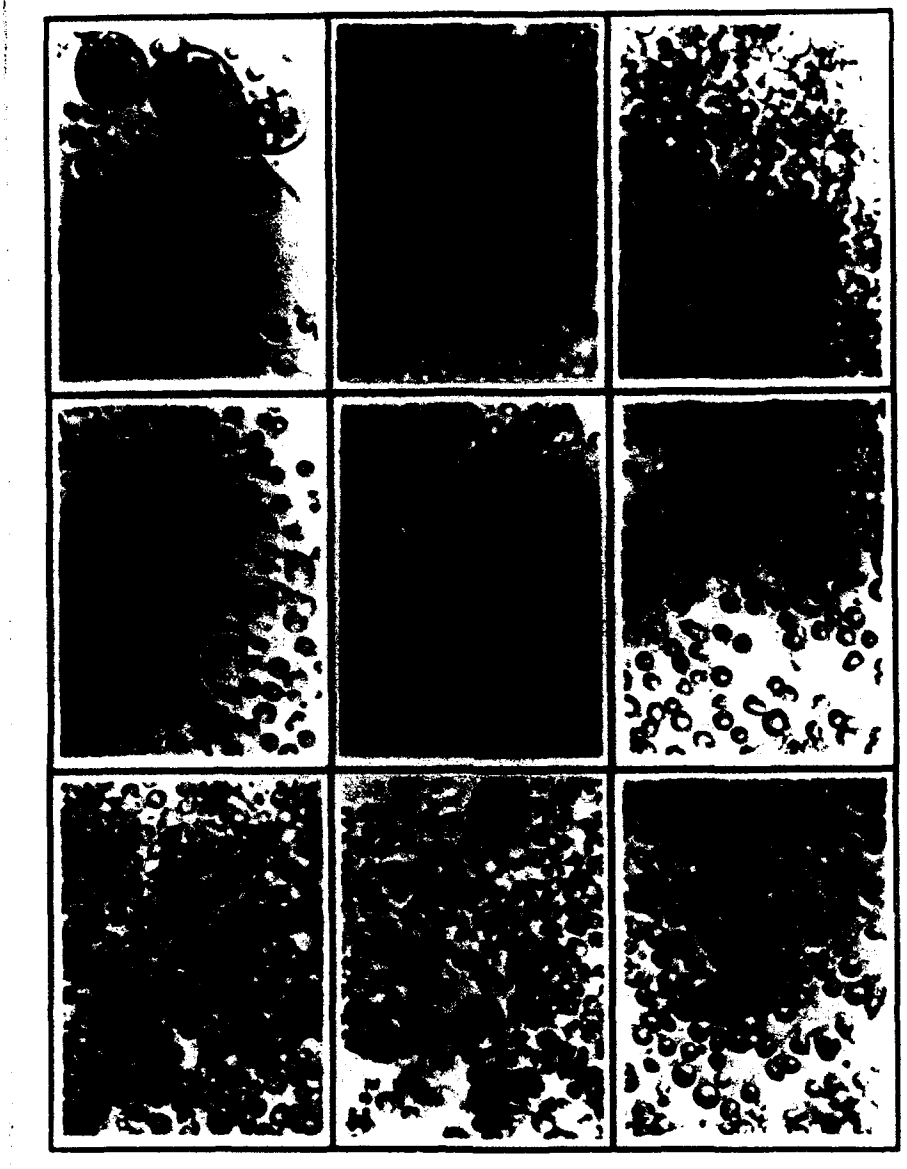
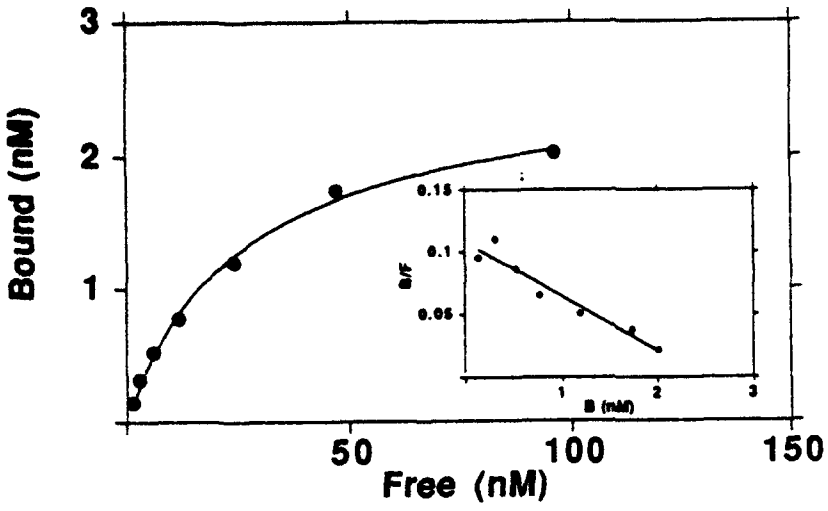


Figure 3: **Micrographs of the inhibition of syncytia formation by various mAbs.**



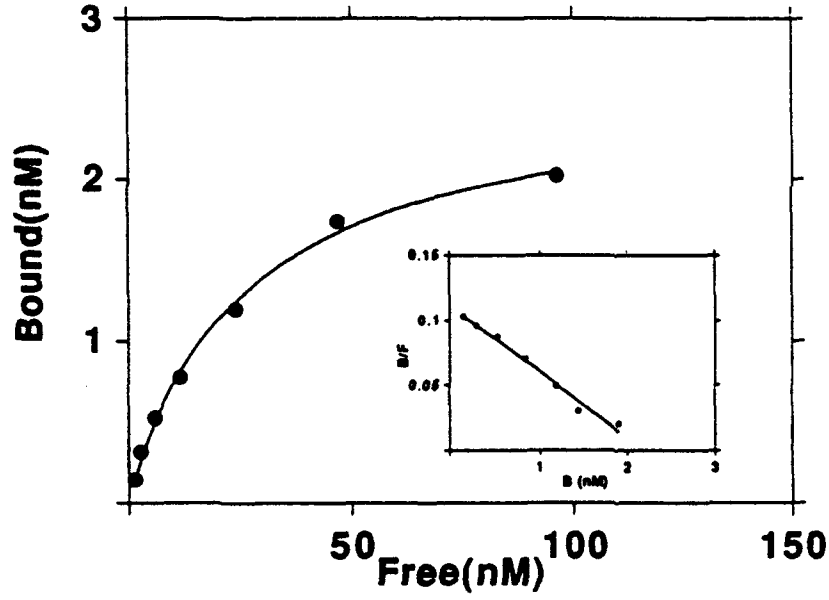
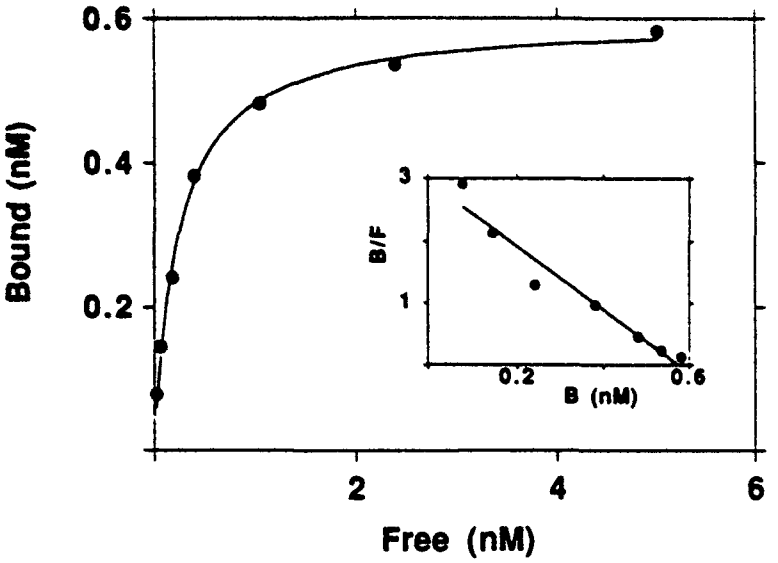


Figure 4: Binding of CG-4 to immobilized CD4 (top) and CD4/gp120 complex (bottom).



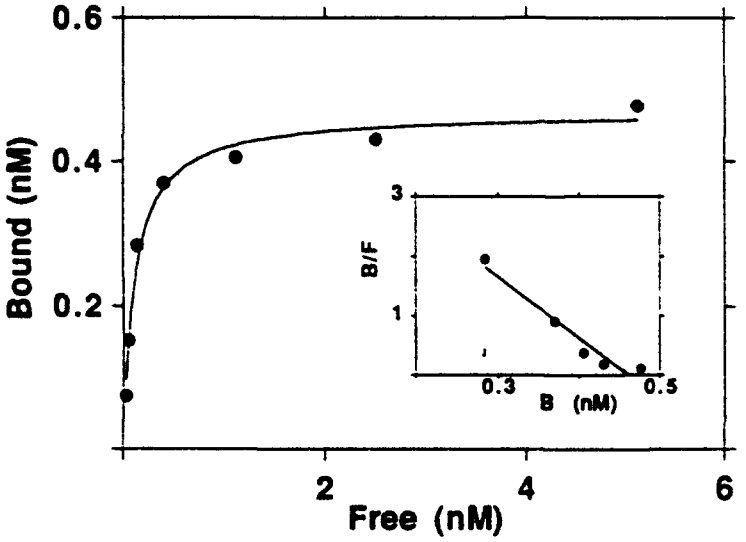


Figure 5: **Binding of CG-76 to immobilized CD4 (top)and CD4/gp120 complex (bottom).**

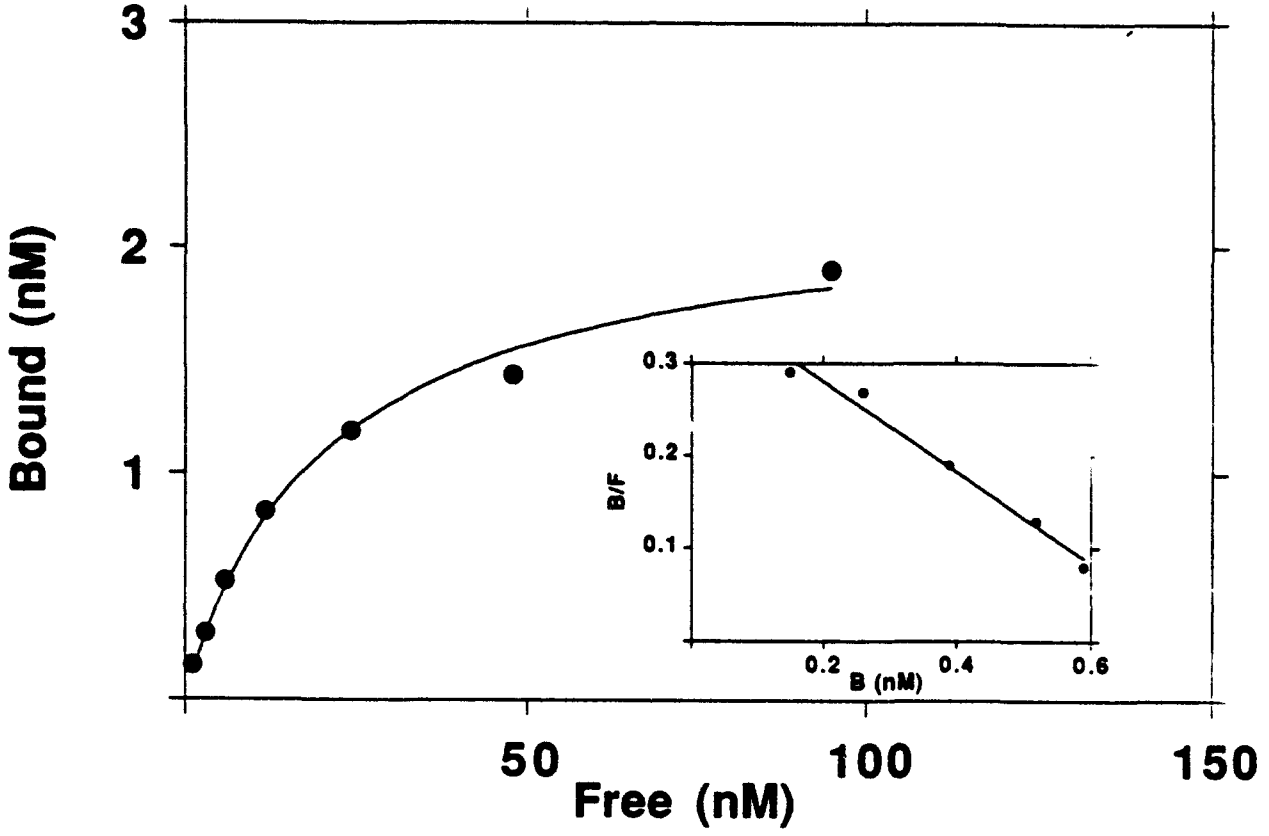


Figure 6: **Binding of CG-10 to immobilized CD4/gp120 complex.**

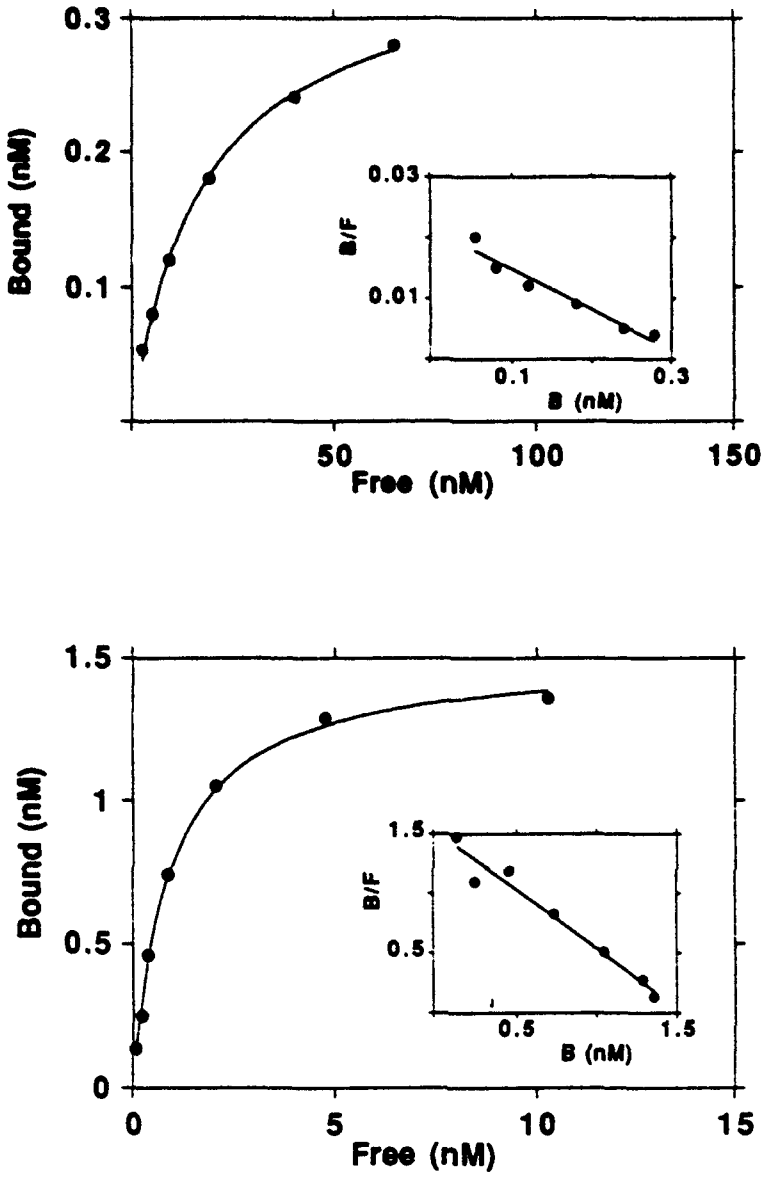


Figure 7: Binding of CG-9 to immobilized CD4 (top) and CD4/gp120 complex (bottom).

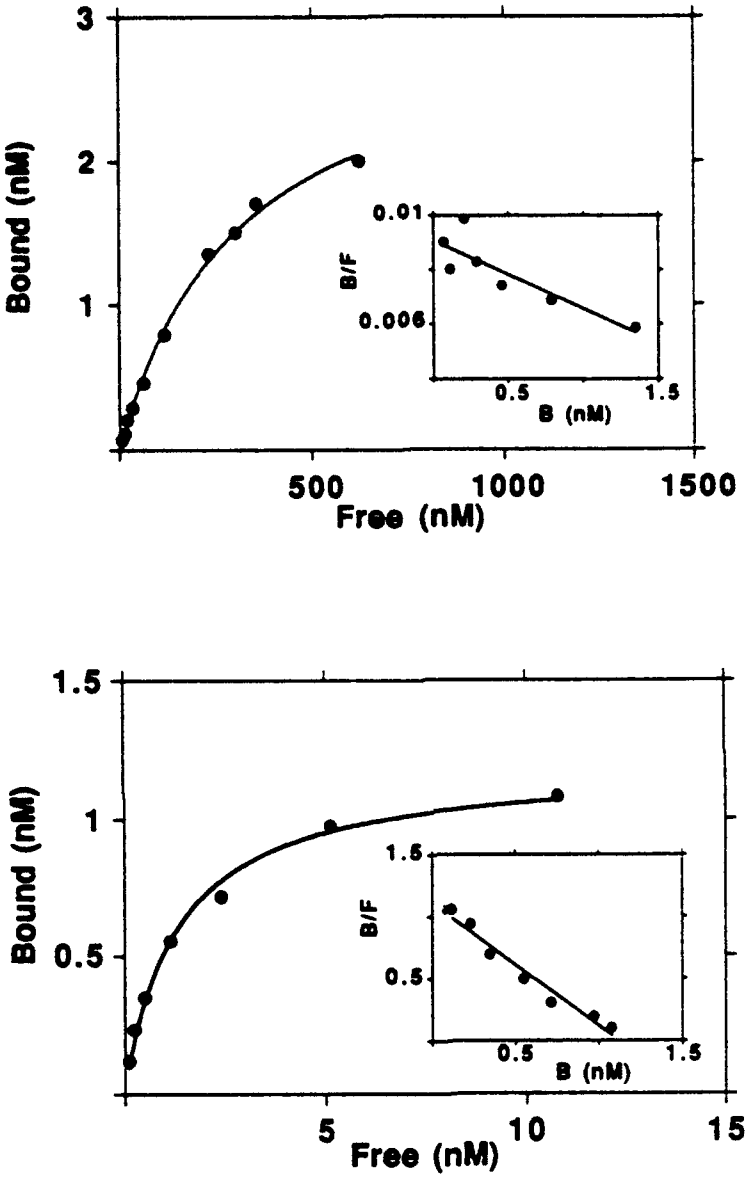


Figure 8: Binding of CG-25 to immobilized CD4 (top) and CD4/gp120 complex (bottom).

In all the assays used CG-10 was persistently complex dependent. On the other hand the extent of amplification as a result of gp120 binding to CD4, seemed to vary for CG-9 and CG-25. It seemed therefore important to establish the effect of gp120 binding to CD4 in a more physiologically relevant system.

Binding of mAbs to cell surface CD4: Clearly the most relevant configuration of CD4 is its cell surface orientation. We therefore used ^{125}I-labeled mAbs to bind CD4 presented on CEM cells in the presence or absence of gp120. In these experiments CG-10 continued to be totally complex dependent (Figure 9). However, the binding of CG-9 and CG-25 actually seemed to be slightly inhibited by gp120. In contrast to this however the binding of CG-1, CG-7 and CG-8 to membrane associated CD4 was markedly enhanced by gp120 (Figure 10). Interestingly, when Scatchard analyses were performed it appeared that the enhancement of binding could be ascribed to two different phenomena. In the case of CG-1 the Bmax of the binding was markedly changed in the presence of gp120 but with no variation in the K_D (Figure 11). This was not the case for CG-7 in which the K_D as well as the Bmax was markedly affected (Figure 12). These results allow the formulation of the explanations presented in Figures 13 and 14.

Serological competitive ELISA: The intent of these experiments was to see whether or not HIV-infected patients had serological activity against the epitopes recognized by the mAbs we had produced. Preliminary analyses of five sera obtained from HIV$^+$ hemophiliacs were analyzed in a competitive ELISA assay. It was found that all five patients could compete for CG-10 and none for CG-4. Lack of competition with the CG-4 was not surprising as the antigen used to produce this clone was recombinant gp120-IIIb. More interesting however, was the fact that the CG-10 epitope appeared to be presented in hemophiliacs.

Further experiments in this direction were performed. We tested some 20 HIV infected patients for the presence of CG-10 like activity in their serum. The assay consisted of using either ^{125}I-labeled CG-10 or biotinylated CG-10. CD4/gp120 was plated on an ELISA plate and then incubated with patient's sera. Afterwards the wells were incubated with the tagged CG-10 and the amount bound was measured. The extent of inhibition was then calculated. Table 6 summarizes a typical experiment.

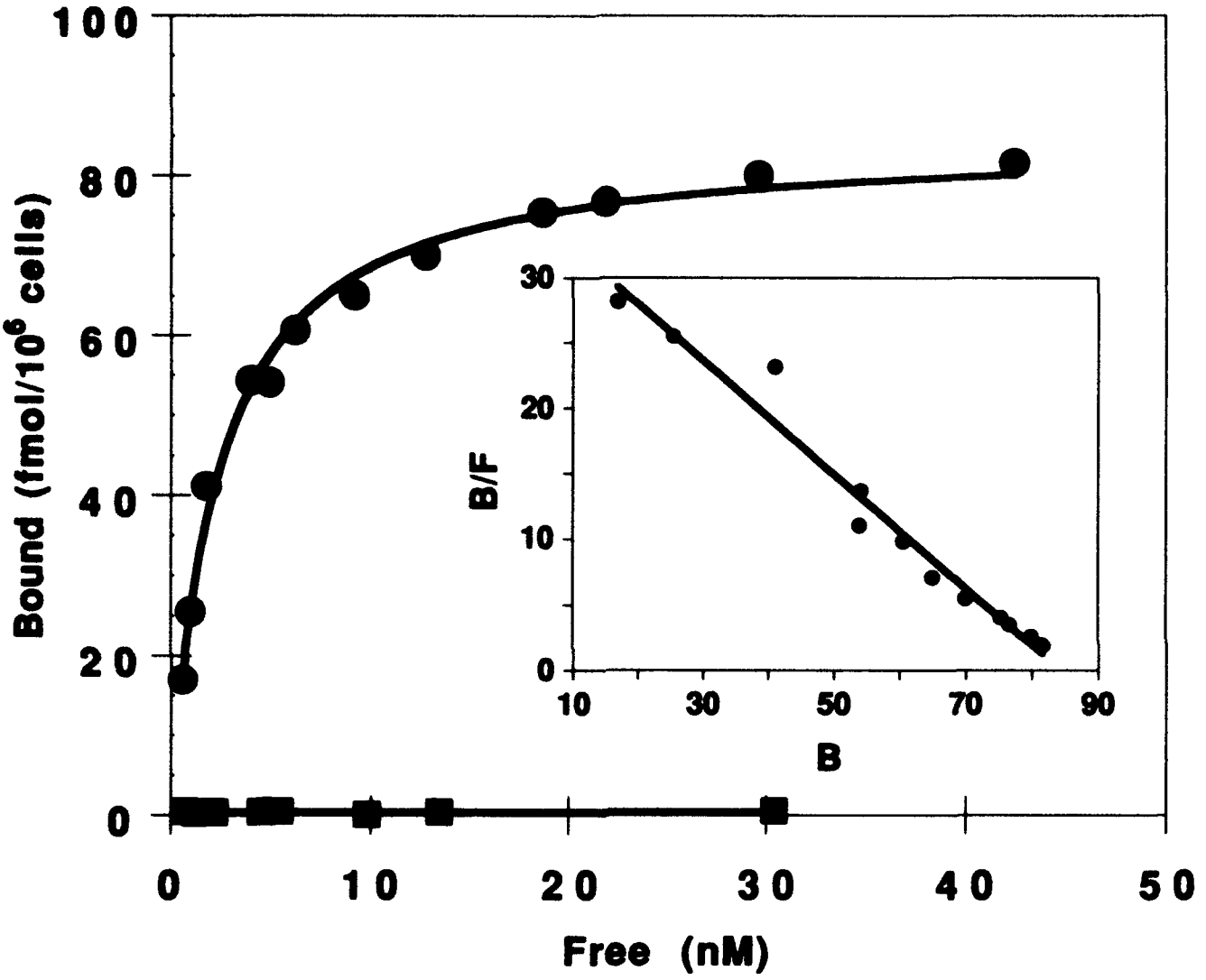


Figure 9: Binding of CG-10 to cell surface CD4/gp120 complex.

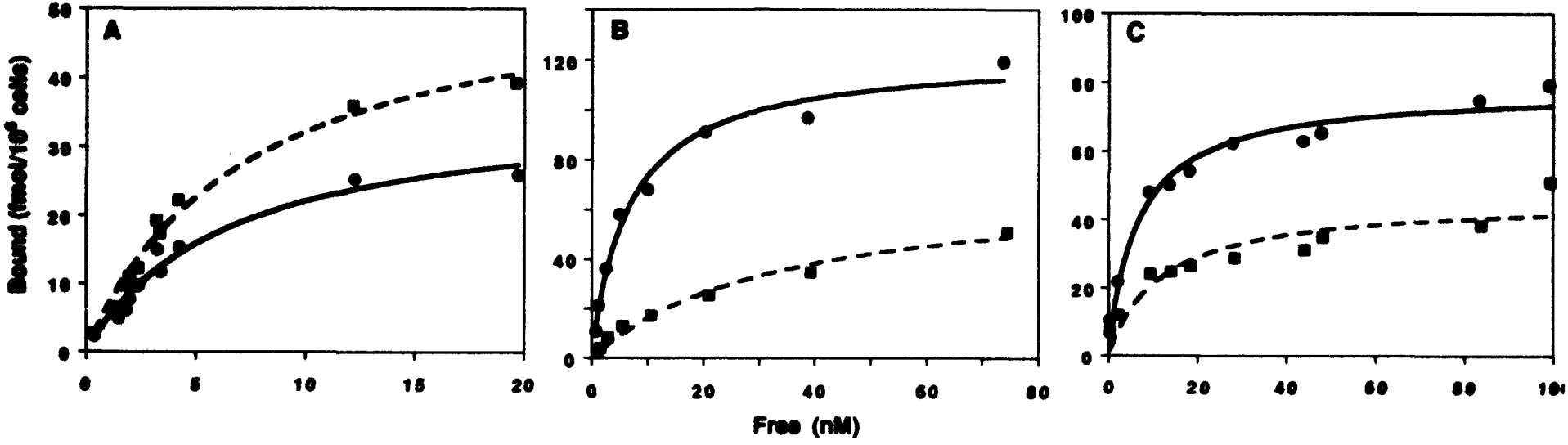


Figure 10: Binding of CG-9 (A), CG-1 (B) and CG-7 (C) to cell surface CD4 (solid) and CD4/gp120 complex (dashed).

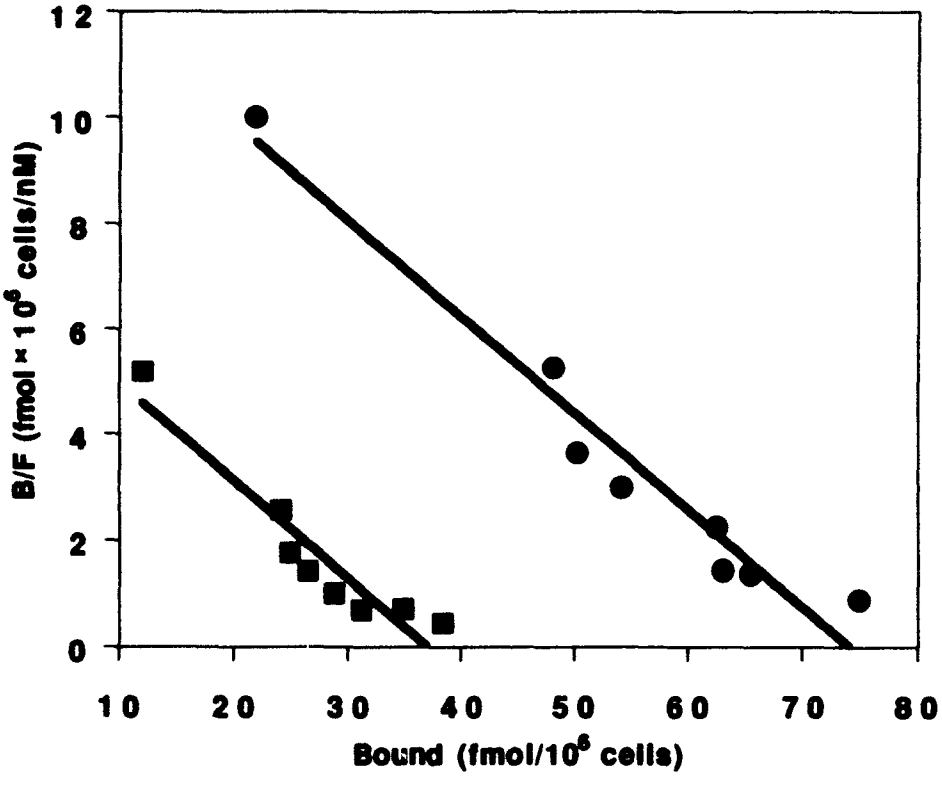


Figure 11: Scatchard analysis od CG-1 binding to cell surface CD4 in the presence (circles) or absence (squares) of gp120.

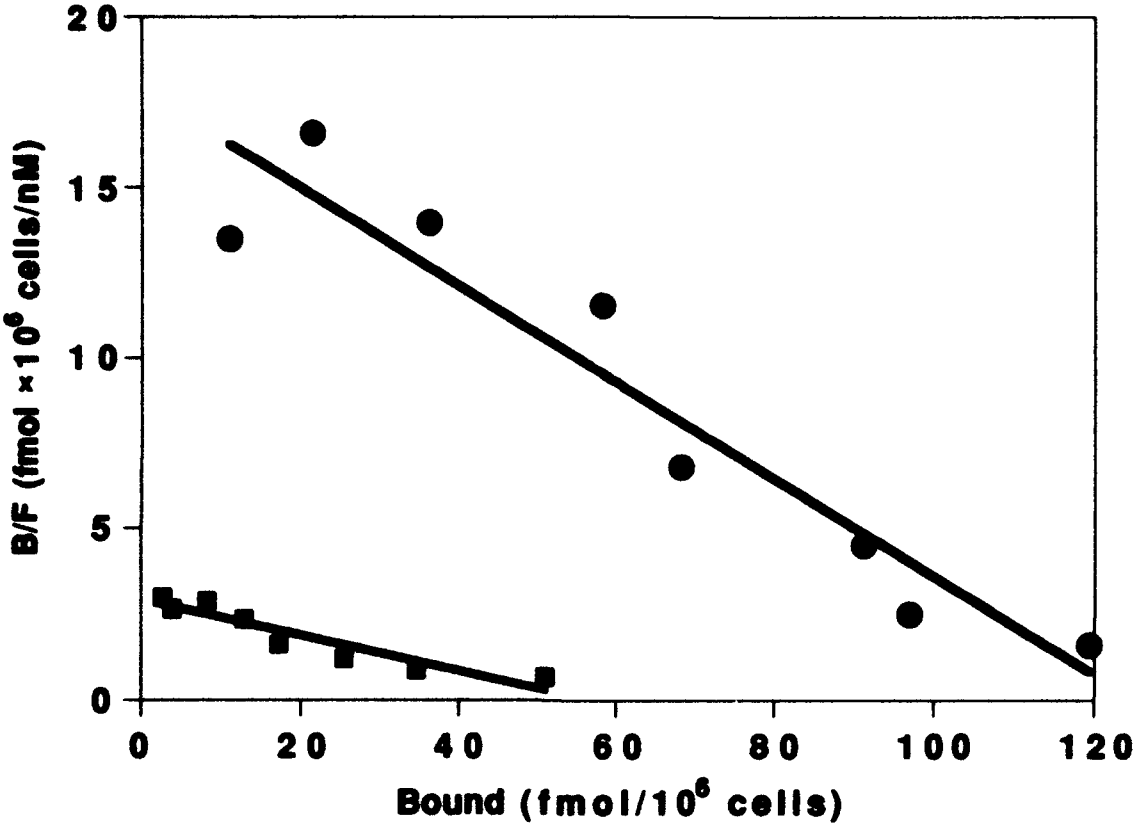
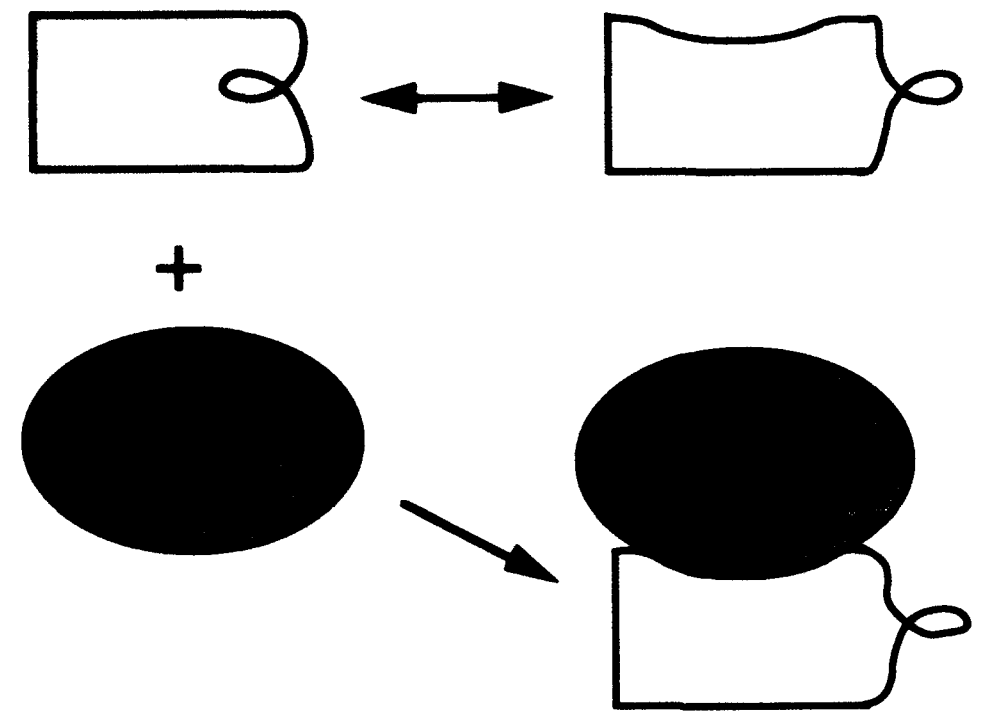


Figure 12: Scatchard analysis od CG-7 binding to cell surface CD4 in the presence (circles) or absence (squares) of gp120.



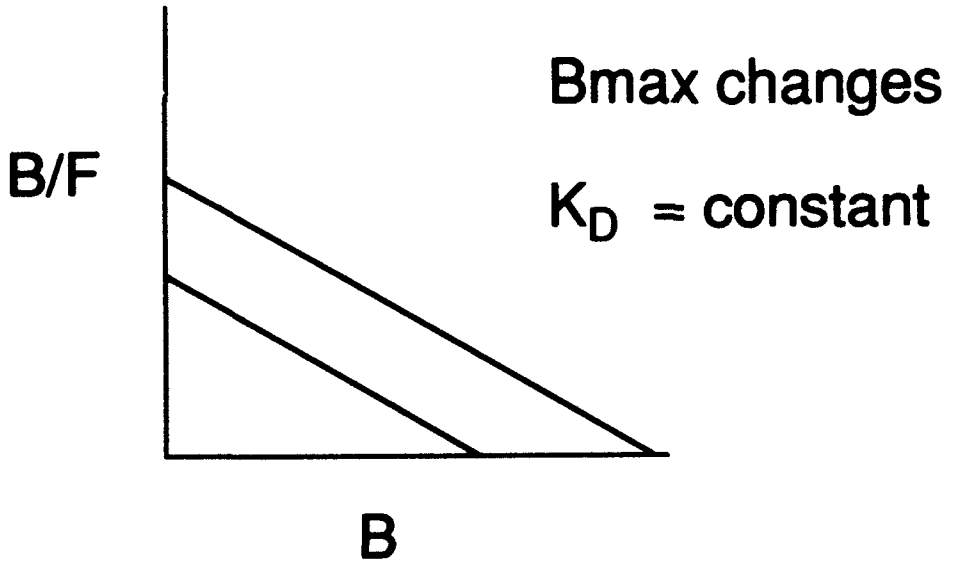


Figure 13: Model for cryptic epitopes. The revelation of the cryptic epitope effects the Bmax while K_D stays constant.

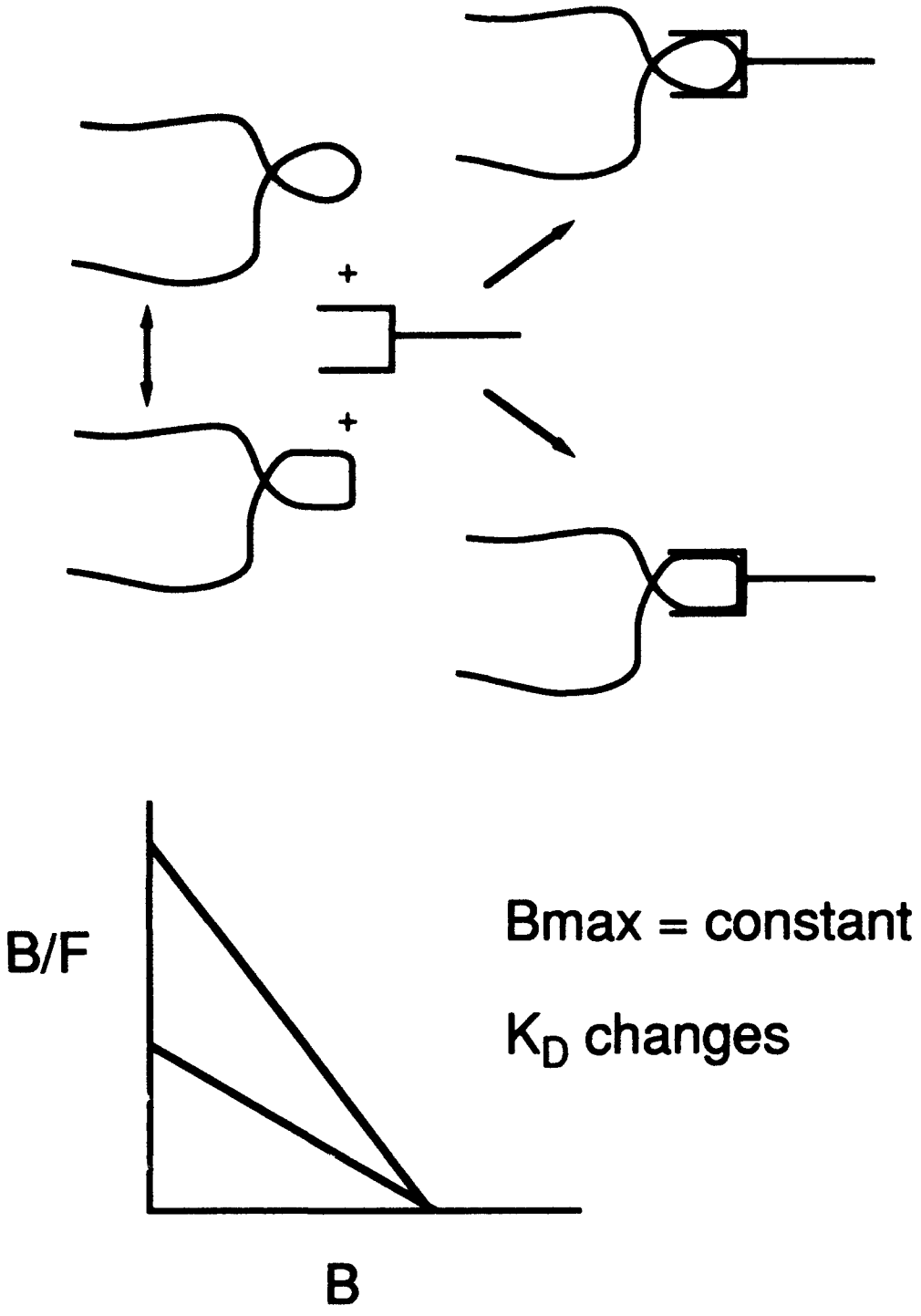


Figure 14: Model for conformational epitopes. The binding of gp120 elicits a conformational rearrangement that causes a better fit of the epitope for the inernal image of the mAb. This is reflected in a genuine decrease in K_D while Bmax can stay constant.

Table 6: Presence of CG-10 activity in HIV infected families:

Serum #	titer	%inhibition	protein mg/ml	%IgG
4139	1/25600	68	74	41
3003	1/25600	64	68	53
3004	1/6400	36	66	44
4167	1/6400	40	-	54
4168	1/25600	77	-	54
4143	1/51200	83	76	54
4142	1/51200	68	74	52
4144	1/25600	75	80	43
4149	1/51200	49	68	36

The coded samples were taken from families in which at least one individual is a HIV infected hemophiliac and the others (wife or child, in two cases two brothers were tested) were infected from that individual.

The medical status of the patients correlates somewhat with the extent of inhibition. However as is shown by the titer value the lower the titer for anti-gp120 activity the lower is the inhibition value. The protein content of the sera samples and the IgG content in each were also determined. The basic conclusion was that at this point the competitive ELISA is not very effective. This is primarily due to the fact that in order to elicit competition rather high amounts (routinely 1/20 - 1/100 dilutions) of serum were used. Under such circumstances many antibodies might bind to the gp120 and simply "coat" and thus obscure the CG-10 epitope of the complex. Thus the greater the general response to gp120 (as is reflected by titer value) the more *apparent* competition but most probably simple steric hindrance.

It would appear that in order to critically evaluate serology it is necessary to map the epitopes and eventually synthesize corresponding peptides and test the direct binding of patient's antibodies to the defined epitopes.

Mapping epitopes: The epitopes of the various mAbs are currently being mapped. This is a difficult problem especially for those mAbs that recognize conformational epitopes. Interestingly, we have made the observation that our soluble CD4 when incubated in urea at 37°C undergoes some proteolytic digestion generating discrete fragments. CG-76 recognized a fragment which is different from that which is bound by CG-9 and CG-25. These analyses have allowed us to determine that CG-76 is most probably beyond the D3 domain of CD4 whereas CG-9 binds to that aspect of CD4 that also binds gp120. Thus the D1-D2 domain of CD4 must harbor the epitope of CG-9. This has been confirmed as we obtained D1-D2 from SmithKline and used it in our blot analyses and ELISA assays. Indeed CG-9 and 25 bind D1-D2 whereas CG-76 does not. In these experiments we have also tested the gp120 association and have found that the gp120/D1-D2 complex is recognized by our complex specific antibodies namely CG-10 and CG-7.

CONCLUSIONS:

The major target of this project focused on the postulate that gp120 changes its conformation upon its association with CD4. The molecular ramification of such induced conformational change would be the appearance of structures that are otherwise hidden, i.e. cryptic epitopes. One could hope that antibodies directed towards these unique structures might interfere with the biology of the gp120 and pathogenesis of HIV.

The experiments performed here illustrated that such epitopes are a reality. The closest results published thus far are those of Celada [9] where he and his colleagues isolated one mAb that could correspond to any one of the three CD4 epitopes that we have identified. Moreover, clone CG-10 is genuinely unique with no parallel reported thus far. This clone is the first and most direct example of a complex dependent mAb as it has excellent affinity for the complex and no detectable binding to CD4 or gp120.

A major task that has been more fully appreciated in the course of this work was the problem of quantitatively characterizing the mAbs that we produced and qualitatively mapping their epitopes. The collaboration with Capt. VanCott proved to be extremely useful for the Tel Aviv laboratory and despite the completion of the contract with USAMRU continuation of collaboration between the two laboratories is much desired.

The critical analyses of binding of mAbs to the cell surface CD4 has allowed us to postulate a model for the revelation of cryptic epitopes as opposed to the conformational rearrangements an thus better fit of epitopes.

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Bibliography, Personnel and Degrees:

Personnel:

The following received pay via this contract:

1. Dr. Galina Denisova
2. Daphna Raviv
3. Diana Buyaner
4. Shoshana Katz
5. Lea Shochat
6. Prof. Jonathan Gershoni

Degrees:

This research is part of the Ph.D. and M.Sc. dergrees of Daphna Raviv and Dianna Buyaner respectively.

Meetings:

Abstracts reporting results from this study were presented:

1. LTCB annual lab meeting 1993
2. LTCB annual lab meeting 1994
3. GSF Schloss Elmau meeting on HIV/membrane interactions November 24-29, 1993 Germany

Publications:

1. Gershoni, J. M., Denisova, G, Raviv D., Smorodinsky, N. I., and Buyaner, D., [1993] HIV binding to its receptor creates specific epitopes for the CD4/gp120 complex. FASEB J. 7:1185.

2. Raviv, D. et al. Conformational transitions of membrane-CD4 associated with the HIV-1 envelope protein gp120. (in preparation)

3. VanCott, T. et al. Characterization of CG-10 binding to HIV-1 infected vs non-infected CD4+ cells. (in preparation).